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Phenotyping Wheat Kernel Symmetry as a Consequence of Different Agronomic Practices

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Abstract: The use of instrumental methods of analysis in the assessment of indices that record changes in symmetry in the structure of grains to evaluate the quality of durum and soft wheat grain is currently considered a search tool that will allow us to obtain previously unavailable data by finding correlations associated with differences in the shape and ratio of starch granules in conditionally symmetrical and asymmetrical wheat fruits (kernels) formed in different field conditions and with different genotypes. Indicators that had previously shown their effectiveness were used to analyze the obviously complex unique material obtained as a result of growing under critically unique sowing conditions in 2022, which affected the stability of grain development and filling. For the evaluation, a typical agronomic comparative experiment was chosen, which was used to evaluate the soil tillage practices (fallow, non-moldboard loosening, and plowing) and sowing dates (early and after excessive rainfalls), which made it possible to analyze a wider range of factors influencing the studied indices. The soil tillage methods were found to affect the uniformity of kernel fullness and their symmetry, and the sowing dates did not lead to significant differences. This study presents detailed changes in the shape of the middle cut of a wheat kernel, associated with assessing the efficiency of kernel filling and the symmetrical distribution of storage substances under the influence of external and internal physical factors that affect the formation of the wheat kernel. The data obtained may be of interest to breeders and developers of predictive phenotyping programs for cereal grain and seeds of other crops, as well as plant physiologists.

Keywords: soft wheat (*Triticum aestivum* L.); hard wheat (*Triticum durum* L.); sowing date; tillage practices; kernel phenotyping; wheat kernel asymmetry; kernel quality

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1. Introduction

Cereal crops such as rice, wheat, and corn are staple foods for a large portion of the world's growing population [1,2]. Wheat consumption is widespread throughout the world, however, mainly in regions such as Europe, part of Asia, and North America. Wheat's popularity is due to the fact that it is rich in proteins and carbohydrates and can also be used to prepare a wide range of food products, including bread, pasta, cookies, and other baked goods [3,4]. Due to the fact that wheat is of significant importance in ensuring global food security for the world's population, it is the focus of research aimed at increasing productivity, nutritional quality, and resistance to environmental changes [4–7]. Increased soil drought, high temperatures, and salinity during the growing season cause significant crop losses that are especially sensitive in regions with unsustainable agriculture.

To produce wheat, highly developed countries mainly use the intensive method, which is based on the abundant use of mineral fertilizers and chemical plant protection products [8,9]. This method allows high crop yields but, at the same time, increases the harmful impact on the environment [8,10,11]. Accordingly, the main directions for the improvement of wheat growing are finding ways to achieve sustainable production with minimal use of chemicals and to obtain high-quality grain for consumers [12].

The quality of wheat grain is a complex concept, since it all depends on whether the grain is suitable for the production of a particular product or not [13]. We can distinguish the external and internal parameters of grain quality. The external quality parameters can be considered the type and purity of color, smell, and absence of damage from diseases and pests [12]. The internal quality parameters include the chemical composition of the single grains (kernels), with special attention to the protein content, moisture content, grinding potential, enzyme activity, and end-use quality [5,12].

To predict the grain quality, many crop models focus primarily on the average kernel size and nitrogen content [14–16]. The programs used for phenotyping grains, such as SmartGrain [17], Grain Scan [18], Win-SEEDLE, SeedCount [19], and SeedCounter [20], take into account the size of the kernel, the color characteristics, and the shape projection parameters. However, these parameters do not reveal the full range of possibilities for predicting the other physical characteristics of the grain and do not take into account the sophisticated asymmetry parameters of a given object. A grain of wheat is by no means a standard ideal object, and is a fruit (caryopsis or kernel) consisting of several elements represented by tissues developed as a result of the functioning of three different genotypes, as follows: the integument is formed by the cells of the mother plant; the embryo is formed by cells of hybrid origin, obtained as a result of the fusion of male and female haploid nuclei; and the endosperm is represented by cells with a triploid set of chromosomes, formed as a result of the fusion of a haploid vegetative nucleus with a mother cell with a diploid set of chromosomes, which is characteristic of double fertilization [21]. Any developmental disorder caused by internal or external physical factors can lead to a decrease in the efficiency and uniformity of the deposition of storage substances, even without disrupting the processes of cell division and tissue formation [22,23]. The physical factors influencing kernel formation are associated with its location in a specific part of the spikelet and ear, as well as with the transport and distribution of the metabolites in each tissue [24]. In this case, various effects of developmental disorders may be observed, such as a decrease in the size of the cells in the tissues and the kernel in general, while maintaining the conditional symmetry of the right and left lobes, as well as disturbances in the development of one of the lobes or a separate zone in both lobes or one of the lobes [25,26]. These changes, which significantly affect the predictability of quality, cannot be recorded using modern methods of either manual or machine processing. It is hoped that, by identifying subtle patterns of changes in indices characterizing fluctuating asymmetry—which is possible only with instrumental analysis—in the future, it will be possible to find simpler approaches for assessment using mass analysis and digitalization [27,28]. Therefore, there is a need to introduce new grain state variables [5]. The size and shape of wheat grains (kernels) is known to affect the processing efficiency (grinding yield and sifting losses) and its value, therefore, small and wrinkled (puny) kernels reduce flour yield [5,29].

The aim of this study was to evaluate the effectiveness of assessing the development of kernels with an emphasis on the criteria used by agronomists by using the example of obviously difficult conditions of climatic collapse in the cultivation region, as follows: the impact of tillage techniques, sowing dates, and varietal characteristics of soft and hard spring wheat on the quality and parameters of kernel shape, characterizing its plumpness (fullness) and shriveling (puniness). Hypothesis 1 assumed that a second sowing period, as well as non-moldboard loosening and fallow, would lead to better grain quality. Hypothesis

2 assumed that the filling of the kernels would be more uniform and symmetrical in the middle kernels.

2. Materials and Methods

2.1. Agrotechnical Conditions

The research was carried out in the Urals region of the Orenburg region. The field experiments were carried out at the experimental site of the Federal Scientific Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences at coordinates 51°77′ N latitude, 55°32′ E longitude.

The soil of the experimental plot is southern carbonate, low-humus, heavy-loamy chernozem. The humus content in the arable soil layer is 3.2–4.0%, total nitrogen—0.20–0.31%, total phosphorus—0.14–0.22%, available phosphorus—1.5–2.5 mg, exchangeable potassium—30–38 mg per 100 g of soil, and pH of the soil solution—7.0–8.1. The lowest moisture capacity in 0–100 cm and 0–150 cm soil layers is 297 mm (27.1%) and 389 mm (25.4%), respectively.

In the field experiments, the reaction of spring soft and spring durum wheat varieties to the backgrounds of basic soil cultivation—plowing and non-moldboard loosening, as well as when sowing in pure fallow—was studied.

The laying of the processing backgrounds was carried out in the fall of the previous year of sowing the varieties. The predecessor was spring wheat. The plowing was carried out with a PN-5-35 plow to a depth of 25–27 cm, non-moldboard loosening with SibIME tines to a depth of 25–27 cm. In the spring, after the snow had melted and the soil had reached sufficient ripeness, harrowing was carried out with BZSS-1.0 tooth harrows across the autumn tillage, and then pre-sowing cultivation with a KPS-4 cultivator to a depth of 8–10 cm was carried out. The preparation of the fallow plot for research purposes consisted of plowing the previous crop with five-time cultivation during the fallow period and loosening at the end of fallow. In the spring, in the year of establishing the experiment on the fallow plot, harrowing and pre-sowing cultivation was carried out before sowing.

The seed sowing rate was 4.0 million viable seeds per hectare. The sowing of the varieties was carried out with a SN-16 seeder, 1.65 m wide, with row spacing of 0.15 m. The seed placement depth was 6 cm. The plot area was 66 sq. m. m (length—40 m and width—1.65 m). In the experiment, zoned varieties of spring soft wheat were sown, as follows: cv. Uchitel (bred at the Orenburg Research Institute of Agriculture, mid-season, growing season 76–86 days); cv. Orenburgskaya 30 (bred at the Federal State Budgetary Institution FSC BST RAS, mid-late, growing season 78–93 days); cv. Ulyanovskaya 105 (bred at Ulyanovsk Research Institute of Agriculture, mid-season, growing season 77–95 days); cv. Tulaikovskaya Zolotistaya (bred in Samara Research Institute of Agriculture, mid-season, growing season 85–95 days); spring durum wheat cultivars Orenburgskaya 10 (mid-season, growing season 70–85 day, bred in Orenburg Research Institute of Agriculture); Bezenchukskaya 210 (mid-season, growing season 75–85 days, bred in Samara Research Institute of Agriculture); Luch 25 (bred in Research Institute of Agriculture of the South-East); and Bezenchukskaya Zolotistaya (mid-season variety, growing season—77–88 days, bred in Samara Research Institute of Agriculture). The experiments were repeated four times. After sowing, rolling with ring rollers KKSh-6 was carried out. Samples for analysis were taken at the stage of complete grain maturity. No fertilizers were applied. No watering was carried out.

Features of the weather conditions of the growing season of 2022 determined the factors that had the greatest influence on the formation of the yield of the studied cultivars. It was in this year that there was a record amount of rainfall that fell immediately after sowing, as follows: 41 mm in the second week and 77 mm in the third week of May (Table S1), resulting in the date of the second sowing being postponed by one month. This is the first time this sowing pattern had developed over the years of experiments in the growing area. Therefore, these results could not be compared with previous growing seasons, and the process of wheat grain phenotyping was limited by the unstable conditions of 2022.

The amount of productive moisture at the first sowing date was 105 mm, and at the second sowing date it was 148 mm. In the heading phase, the amount of available moisture in the 1-m soil layer was 46 mm at the first sowing date and 76 mm at the second sowing date. By the end of the growing season, the amount of productive moisture in the 1-m layer of soil was 5 mm at the first sowing date and 12 mm at the second sowing date. Weather conditions can influence the duration of the stages of plant ontogenesis but are generally determined by the genotype. In this experiment, we controlled the duration of the stages depending on the tillage practice (Table S2).

2.2. Preparation of Ears and Kernels for Analysis

The obtained wheat ears were scanned using a ruler on a professional Epson Perfection V550 Photo scanner. After this, each ear was disassembled into simple spikelets, maintaining the order of the spikelets on the main axis, which was subsequently disassembled into individual kernels. The location of the kernels in a simple spikelet was designated as left, middle, and right. Next, they were recorded on an A4 sheet of paper with adhesive tape, indicating the number of complex ears and the number of simple spikelets. When indicating the left and right kernels, the simple spikelet was oriented so that the place of attachment of the simple spikelet to the common axis (rachis) was the back side. Therefore, the left kernel of the front side was designated as the left kernel under the analysis. Next, in the ImageJ program [30], for each ear, the length of the ear without awns was determined with an accuracy of 0.01 mm, and the number of spikelets, the number of fertile spikelets, the number of kernels, the number of middle kernels, the number of immature kernels, and the number of affected kernels were calculated from the photo. A total of 260 ears of wheat were examined, according to the schema (Figures S1 and S2).

2.3. Sampling and Preparation of Kernel Cuts for Analysis

After all of the kernels were removed from the ears, and the number of left, right, and middle kernels was counted. Next, the method of randomized data selection was used, as described in our earlier works [25], implemented in the statistical program SPSS Statistics 25. The algorithm consisted of using a random number generator built into SPSS Statistics. In the calculations, we proceeded from the preliminary data, assuming them from a sample of 90 left kernels (for example, on average, about 90 left kernels can be extracted from 10 ears), therefore, strictly 15 kernels were randomly selected from this quantity. In addition, in the calculations in the program, the kernels included in the sample were taken into account, indicating the number of ears and the number of spikelets. In a similar way, 15 left, 15 right, and 15 middle kernels were selected. The calculation that a kernel sample size of 15 is sufficient was based on an earlier study [22]. If the sample contained less than 15 medium kernels, all available kernels were used for analysis.

The selected kernels, indicating the code (ear number, spikelet number, and location in the spikelet), were transferred to a new sheet with adhesive tape, having previously measured the mass of each kernel on an analytical balance (Sartorius, Gottingen, Germany) with an accuracy of 0.0001 g. Next, we scanned the samples with a ruler on an Epson Perfection V550 Photo scanner with an image resolution of 600 dpi. In the ImageJ program, the kernel parameters were measured with an accuracy of 0.001, as follows: area in mm^2, perimeter in mm, and length and width in mm. After this, each core was cut in the middle and secured with adhesive tape, so that the cross section was directed facing straight up. The orientation in space of all kernels was the same, so that it was possible to compare the left and right sides of the kernel relative to the axis of symmetry passing through the junction of the two halves of the kernel. Using the same scanner, we obtained an image with a ruler with an extension of 600 dpi. Then, in ImageJ, using the method described by Aniskina et al. (2023) [22], the depth of the kernel (the length of the axis of symmetry), the length of the perpendiculars from the tops of the endosperm to the axis of symmetry, the length of the perpendiculars along the widest part of the kernel to the axis of symmetry, the length of the perpendiculars to the edges of the base of the central hole, the length of the perpendiculars from the edge of the kernel to the axis of symmetry under the central hole, the length of the section of the axis of symmetry from the bottom of the kernel cut to the central hole, and the length of the perpendiculars to the angle between the previous two segments were measured with an accuracy of 0.001 mm in the kernel,. Using the above method, the value of the asymmetry of the kernels was calculated as the arithmetic mean of the indices of the relative asymmetry of the kernels along the above perpendiculars. About 1170 kernels were described in this way.

2.4. Hyperspectrum Imaging

To obtain hyperspectral images, a Synergotron Fenoscaner (Institute of Development Strategy, Moscow, Russia) was used, consisting of a hyperspectral camera with scanning technology, a separation module with a transmission grating with high diffraction efficiency, and a camera based on a high-sensitivity matrix. This composition of the research complex allowed us to solve complex problems characteristic of conventional hyperspectral cameras, such as the external mechanism of longitudinal scanning and complex focus.

Spectral range: 400–1032 nm, spectral resolution above 2.5 nm, with 300 spectral channels. Image resolution: 1920 × 1920. Every 10 measurements, calibration measurements were taken on a white calibration panel coated with $BaSO_4$.

The image analysis was carried out using Gelion software (Moscow, Russian Federation, https://gelion.agro.msu.ru/, accessed on 1 March 2024). An analysis of the spectral curve approximation of kernel images compared to the reference values of the spectral patterns of the kernel coloring substances—in this case, anthocyanins (AnC)—was carried out. Patterns were identified by the type of spectral curve, which displayed the dependence of spectral brightness on the wavelength. At the same time, the reference spectra were brought to a single brightness scale—reflection coefficients. The reflectance coefficient (r) is the ratio of reflected radiation to incident radiation in the direction perpendicular to the surface. The reflectance in a certain direction relative to the earth's surface is called the spectral albedo.

2.5. Statistical Analysis

Testing for normality of distribution was performed using the Kolmogorov–Smirnov method in SPSS Statistics 25 (the software used was SPSS Statistics 25). The differences between the research options were established using the Shidak criterion in two-factor analysis of variance and the *t*-test method with Livigne's test for equality of variances for paired independent samples when comparing the perpendiculars of the kernel cut. Confidence intervals for arithmetic means are given, with the standard deviation indicated. The presence of correlations was established using the Pearson method with two-sided testing at the 0.01 and 0.05 significance level.

3. Results

Methodological approaches to assessing technological solutions and forecasting methodology are currently becoming increasingly relevant, since predictability is a determining factor in the profitability of farms in the unsustainable agriculture zone. In this work, new criteria for assessing the morphological characteristics of grains were applied, previously proposed for wheat considering its botanical and physiological characteristics [22,25] using two-factor analysis of variance. The results are given taking into account the varietal differences, as well as the method of tillage and sowing dates.

3.1. Effects of Tillage Practices on the Main Parameters of the Wheat Ear as a Result of the First Date of Sowing

The impact of tillage practices on the main parameters of the ear of different cultivars of wheat sown in the first period has been revealed. The two-factor analysis of variance showed that the factor "cultivar" influenced all of the parameters, while the factor "tillage practice" affected only the length of the ear (without taking into account the awns), the number of spikelets, and the number of immature kernels. The combination of factors did not affect the number of immature kernels (Table 1).

Table 1. The results of the *p*-value analysis of variance on the influence of the cultivar, tillage practice (fallow, plowing, and non-moldboard loosening), and the combination of these factors on the characteristics of the ears and kernels.

Dependent Variable (Attribute Characteristic)	Independent Variables (Influence of Factors)		
	Cultivar	Tillage Practice	Factors Combination (Cultivar and Tillage Practice)
Ear length without awns, mm	<<0.005 *	<<0.005 *	<<0.005 *
Spikelet number, pcs	0.001 *	<<0.005 *	0.003 *
Fertile spikelet number, pcs.	<<0.005 *	0.943	<<0.005 *
Kernel number, pcs.	<<0.005 *	0.274	<<0.005 *
Immature kernel number, pcs.	<<0.005 *	0.005 *	0.087
Affected kernel number, pcs.	0.002 *	0.178	0.029 *
Middle kernel number, pcs	<<0.005 *	0.114	0.016 *

Asterisk indicates significance at $p = 0.05$. Abbreviation: pcs—pieces.

Thus, the length of the ear without awns in the cvs. Uchitel, Orenburgskaya 30, and Ulyanovskaya 105, sown after plowing, had a significantly larger size compared to this indicator in the same cultivars sown with other tillage practices (Table 2). However, in terms of the number of kernels in the cv. Ulyanovskaya 105, the accessions in the tillage practices with non-moldboard loosening and fallow stand out. Pearson's correlation analysis did not reveal a significant relationship between the length of the ear without awns and the number of kernels for cv. Ulyanovskaya 105.

The cv. Uchitel, when using non-moldboard loosening, demonstrated a high number of fertile spikelets and kernel yield with the least disease damage. The cv. Uchitel had the best results in such indicators as ear length without awns and the number of fertile spikelets after plowing but showed significantly severe damage to the kernels compared to the other cultivars. Thus, non-moldboard loosening and fallow resulted in the better "protection" of the kernels of the cv. Uchitel from damage.

The cv. Orenburgskaya 10, compared to the other varieties, showed the lowest indicators of the studied traits. A slightly larger number of kernels can be achieved when growing the cultivar after fallow (17.5 $\pm$ 6.5 pieces versus 10.6 and 7.5 pieces with non-moldboard loosening and plowing, respectively).

The cvs. Bezenchukskaya 210 and Tulaikovskaya Zolotistaya showed the largest number of middle kernels among all of the cultivars when sowing after non-moldboard loosening, while retaining the maximum values for the number of kernels in an ear and the number of fertile spikelets. Fallow also had a beneficial effect on the listed traits.

In the experiment with plowing in cvs. Orenburgskaya 30 and Tulaikovskaya Zolotistaya, a large number of immature kernels was noted.

Table 2. Average values of parameters of the wheat ear, spikelets, and kernels after various tillage practices at the first sowing date.

Cultivar	Tillage Practice	Ear Length without Awns, mm	Spikelet Number, pcs	Fertile Spikelet Number, pcs	Kernel Number, pcs	Immature Kernel Number, pcs	Affected Kernel Number, pcs	Middle Kernel Number, pcs
Uchitel	Plowing	86.5 ± 13.2 f	14.5 ± 2.5 b,c	11.2 ± 3.2 d	19.7 ± 8.8 c	2.6 ± 1.8 a	2.9 ± 1.4 c	3.4 ± 3.0 a,b
	Non-moldboard loosening	68.9 ± 5.3 c,d	12.9 ± 0.7 a,b,c	11.1 ± 1.7 d	18.4 ± 4.3 c	2.5 ± 3.0 a	1.9 ± 1.6 a,b	0.8 ± 1.4 a
	Fallow	63.8 ± 12,7 b,c	12.0 ± 2.7 a	7.2 ± 2.9 a,b	10.8 ± 4.4 a,b,c	2.3 ± 1.6 a	0.4 ± 0.7 a,b	0.2 ± 0.6 a
Ulyanovskaya 105	Plowing	74.9 ± 5.4 d,e,f	14.8 ± 1.4 c	11.8 ± 2.5 d	19.5 ± 7.9 c	3.1 ± 1.9 a	1.1 ± 1.3 a,b	2.3 ± 1.9 a,b
	Non-moldboard loosening	71.5 ± 11.5 d,e	14.0 ± 2.2 b,c	11.8 ± 2.3 d	21.0 ± 8.3 d	1.0 ± 1.2 a	0.9 ± 0.7 a,b	2.8 ± 3.0 a,b
	Fallow	74.5 ± 5.6 c,d,e	13.8 ± 1.2 b,c	12.2 ± 1.2 d	22.4 ± 3.0 d	1.2 ± 1.9 a	0.3 ± 0.7 a,b	1.8 ± 1.9 a,b
Orenburgskaya 10	Plowing	60.2 ± 9.1 b,c	13.9 ± 2.0 b,c	5.1 ± 2.8 a	7.5 ± 5.4 a	0.9 ± 1.0 a	0.1 ± 0.3 a	0.8 ± 0.8 a,b
	Non-moldboard loosening	46.1 ± 5.6 a	10.3 ± 1.2 a	6.3 ± 1.3 a	10.6 ± 2.5 a,b	0.5 ± 0.7 a	0.4 ± 0.5 a,b	1.0 ± 0.9 a,b
	Fallow	59.5 ± 7.9 b	13.6 ± 1.8 b,c	9.8 ± 1.9 b,c	17.5 ± 6.5 c	1.8 ± 1.4 a	1.2 ± 1.3 a,b	1.5 ± 1.9 a,b
Bezenchukskaya 210	Plowing	60.1 ± 4.9 b,c	14.6 ± 1.6 b,c	10.8 ± 2.7 d	16.3 ± 5.3 c	1.2 ± 1.1 a	1.1 ± 1.4 a,b	1.0 ± 1.4 a,b
	Non-moldboard loosening	52.4 ± 6.1 a,b	12.5 ± 1.6 a,b	9.9 ± 1.9 c,d	21.8 ± 7.9 d	1.2 ± 0.9 a	1.1 ± 0.9 a,b	4.1 ± 5.0 b
	Fallow	51.4 ± 5.7 a	14.6 ± 1.3 b,c	11.0 ± 2.0 d	21.8 ± 5.7 d	1.6 ± 1.8 a	1.3 ± 1.8 a,b	2.7 ± 2.2 a,b
Orenburgskaya 30	Plowing	80.4 ± 7.5 e,f	14.2 ± 1.3 b,c	11.4 ± 2.2 d	21.6 ± 4.9 d	4.5 ± 3.6 b	1.0 ± 1.2 a,b	3.0 ± 2.2 a,b
	Non-moldboard loosening	66.7 ± 5.9 c,d	12.7 ± 1.2 a,b,c	10.5 ± 1.4 c	19.2 ± 2.9 a,b	2.5 ± 1.2 a	2.6 ± 2.8 b,c	3.0 ± 1.1 a,b
	Fallow	65.7 ± 4.5 c,d	12.9 ± 1.0 b,c	9.9 ± 0.9 c,d	17.6 ± 2.3 c	1.9 ± 1.4 a	1.4 ± 1.8 a,b	1.1 ± 1.3 a,b
Tulaikovskaya Zolotistaya	Plowing	72.8 ± 8.3 c,d,e	13.3 ± 1.2 b,c	10.2 ± 2.5 c,d	18.6 ± 6.6 c	4.3 ± 1.6 b	0.3 ± 0.7 a,b	3.5 ± 2.5 a,b
	Non-moldboard loosening	66.2 ± 5.9 c,d	12.5 ± 1.2 a,b,c	10.9 ± 1.9 d	21.8 ± 6.1 d	2.7 ± 2.2 a	0.9 ± 1.4 a,b	4.3 ± 3.2 b
	Fallow	67.1 ± 12.5 c,d	13.0 ± 1.6 b,c	11.1 ± 1.9 d	21.2 ± 6.2 d	2.2 ± 2.6 a	0.0 ± 0.0 a	3.5 ± 2.5 a,b

The letters indicate significant differences between the indicators, established with Shidak post hoc test for multiple comparisons (p = 0.05). The maximum (warm tint) and minimum (cold tint) values of the characteristics are highlighted in color. Blue color means the minimum average value of the feature; orange color indicates the maximum average value of the feature. Abbreviation: pcs—pieces.

3.2. Influence of Factors on the Main Ear Parameters under Two Dates of Wheat Sowing

An evaluation of the influence of factors on the ear main indicators in the cvs. Ulyanovskaya 105 and Orenburgskaya 10 (experiment with plowing) showed that the cultivar choice affects five indicators out of seven (Table 3). The sowing date was significant for the number of fertile spikelets, the number of kernels, the number of immature kernels, and the number of middle kernels. The combination of factors was significant for the number of fertile spikelets, the total number of kernels, and the number of immature kernels.

Table 3. Results of analysis of variance under assessing the influence of wheat cultivar, two sowing dates, and a combination of these factors on the characteristics of the ears and kernels when sowing after plowing.

Dependent Variable (Trait)	Independent Variables (Influence of Factors)		
	Cultivar	Sowing Date	Combination of Factors (Cultivar and Sowing Date)
Ear length without awns, mm	<<0.005 *	0.543	0.196
Spikelet number, pcs	0.087	0.129	0.918
Fertile spikelet number, pcs.	<<0.005 *	<<0.005 *	<<0.005 *
Kernel number, pcs.	<<0.005 *	<<0.005 *	<<0.005 *
Immature kernel number, pcs.	0.004 *	<<0.005 *	0.004 *
Affected kernel number, pcs.	0.003 *	0.149	0.229
Middle kernel number, pcs	0.800	<<0.005 *	0.210

Two cvs. Ulyanovskaya 105 and Orenburgskaya 10 were evaluated under two sowing dates with a difference of one month. Asterisk above the p-value indicates a significant influence of the factor at p = 0.05. Abbreviation: pcs—pieces.

The cv. Orenburgskaya 10 had low indicator values when sown on the first date (Table 4). In addition, when sowing this cultivar on the second date, a significantly larger number of fertile spikelets, total number of kernels, and number of middle kernels were recorded.

The cv. Ulyanovskaya 105 showed a significantly higher total number of kernels and number of middle kernels in the second sowing date, while the number of damaged and immature kernels was reduced.

Table 4. Average values of ear, spikelet, and kernel characteristics of two wheat cultivars at two sowing dates after plowing.

Cultivar	Sowing Date	Spike Length without Awns, mm	Spikelet Number, pcs	Fertile Spikelet Number, pcs.	Kernel Number, pcs.	Immature Kernel Number, pcs.	Affected Kernel Number, pcs.	Middle Kernel Number, pcs
Ulyanovskaya 105	First	74.9 ± 5.4 b	14.8 ± 1.4 a	11.8 ± 2.5 b	19.5 ± 7.8 b	3.1 ± 1.8 b	1.1 ± 1.2 b	2.3 ± 1.9 a
	Second	76.4 ± 7.3 b	14.0 ± 1.4 a	13.6 ± 1.2 b	30.2 ± 4.9 c	0.3 ± 0.7 a	0.5 ± 0.7 a,b	6.3 ± 3.3 b
Orenburgskaya 10	First	60.2 ± 9.1 a	13.9 ± 2.0 a	5.1 ± 2.8 a	7.5 ± 5.4 a	0.9 ± 0.9 a	0.1 ± 0.3 a	0.8 ± 0.8 a
	Second	56.1 ± 3.8 a	13.2 ± 1.6 a	12.8 ± 1.4 b	30.2 ± 7.1 c	0.3 ± 0.5 a	0 a	7.3 ± 4.8 b

The letters indicate significant differences between the indicators, established with Shidak post hoc test for multiple comparisons (p = 0.05). The maximum (warm tint) and minimum (cold tint) values of the characteristics are highlighted in color. Blue color means the minimum average value of the feature; orange color indicates the maximum average value of the feature. Abbreviation: pcs—pieces.

When wheat cvs. Bezenchukskaya Zolotistaya and Luch 25 were sown after fallow on two dates with a difference of one month, it was revealed that the factor "cultivar" was significant for both the total number of kernels and the number of middle kernels; moreover, the factor "sowing date" was important for the number of spikelets, the number of immature kernels, and the number of middle kernels (Table 5). The combination of factors was significant for the number of spikelets and the number of middle kernels.

Table 5. Results of analysis of variance under assessing the influence of wheat cultivar, two sowing dates, and a combination of these factors on the characteristics of the ears and kernels when sowing after fallow.

Dependent Variable (Attribute Characteristic)	Independent Variables (Influence of Factors)		
	Cultivar	Sowing Date	Combination of Factors (Cultivar and Sowing Date)
Ear length without awns, mm	0.468	0.619	0.959
Spikelet number, pcs	0.140	<<0.005 *	<<0.005 *
Fertile spikelet number, pcs.	0.381	0.059	0.294
Kernel number, pcs.	0.040 *	0.977	0.114
Immature kernel number, pcs.	0.620	0.002 *	0.620
Affected kernel number, pcs.	0.186	0.186	0.980
Middle kernel number, pcs	0.036 *	0.010 *	0.004 *

Two cvs. Bezenchukskaya Zolotistaya and Luch 25 were evaluated under two sowing dates with a difference of one month. Asterisk above the p-value indicates a significant influence of the factor at p = 0.05. Abbreviation: pcs—pieces.

When sowing the cv. Luch 25 on the second date, it was found that the number of immature kernels significantly decreased (Table 6), while, for the other indicators, there were no differences between the sowing dates. Sowing the cv. Bezenchukskaya Zolotistaya on the first date resulted in the formation of a larger number of spikelets, however, the number of immature kernels also increased. The second sowing date led to an increase in the number of middle kernels.

Table 6. Average values of ear, spikelet, and kernel characteristics of two wheat cultivars at two sowing dates after fallow.

Cultivar	Sowing Date	Ear Length without Awns, mm	Spikelet Number, pcs	Fertile Spikelet Number, pcs.	Immature Kernel Number, pcs.	Immature Kernel Number, pcs.	Affected Immature Kernel Number, pcs.	Middle Immature Kernel Number, pcs.
Luch 25	First	48.8 ± 7.4 a	12.9 ± 1.4 a	10.8 ± 1.5 a	20.6 ± 5.5 a	1.7 ± 2.0 b	0.7 ± 0.8 a	2.5 ± 2.3 a
	Second	49.8 ± 5.2 a	12.9 ± 1.5 a	10.3 ± 2.0 a	17.7 ± 5.1 a	0.2 ± 0.4 a	0.4 ± 0.8 a	2.2 ± 1.4 a
Bezenchukskaya Zolotistaya	First	50.4 ± 9.9 a	15.2 ± 1.2 b	11.9 ± 2.3 a	21.5 ± 6.2 a	1.3 ± 1.4 b	0.4 ± 0.7 a	1.8 ± 1.8 a
	Second	51.9 ± 3.6 a	11.8 ± 0.8 a	10.2 ± 0.8 a	24.3 ± 5.4 a	0.2 ± 0.4 a	0.1 ± 0.3 a	6.2 ± 3.4 b

The letters indicate significant differences between the indicators, established with Shidak post hoc test for multiple comparisons (p = 0.05). The maximum (warm tint) and minimum (cold tint) values of the characteristics are highlighted in color. Blue color means the minimum average value of the feature; orange color indicates the maximum average value of the feature. Abbreviation: pcs—pieces.

3.3. Descriptive Statistics of Kernel Weight

The tillage practice did not have a significant effect on the kernel weight of the cvs. Uchitel, Orenburgskaya 10, Bezenchukskaya 210, Orenburgskaya 30, or Tulaikovskaya Zolotistaya (Figure 1). However, in the cv. Ulyanovskaya 105, the kernels from the plants sown after fallow had a greater weight than the kernels from the plants sown after plowing. In general, the cv. Tulaikovskaya Zolotistaya, when sown after plowing, and the Uchitel variety, when sown after fallow, showed small kernels (average weight of kernels: 0.0250 g and 0.0274 g, respectively). Some cultivars, after certain tillage practices, showed kernels with an increased weight, as follows: Orenburgskaya 10 after fallow (0.0367 g), Bezenchukskaya 210 after non-moldboard loosening (0.0407 g), Ulyanovskaya 105 after fallow (0.0415 g), and Bezenchukskaya 210 after fallow (0.0423 g).

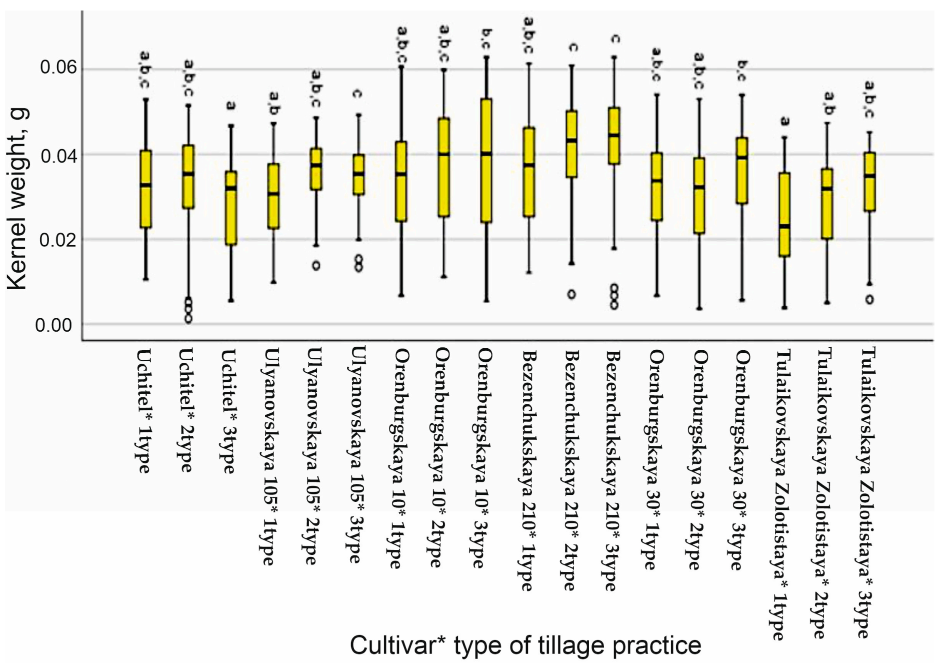


Figure 1. Descriptive statistics of the kernel weight in different cultivars of wheat in the first sowing date with three types of tillage practice, where type 1 is plowing, type 2 is non-moldboard loosening, and type 3 is fallow.

The analysis of variance confirmed the significant influence of the cultivar and sowing date on the kernel weight, but there was no influence of the combination of these factors (Figure 2). In the second date of sowing after plowing, kernels of greater weight were formed in the cvs. Ulyanovskaya 105 and Orenburgskaya 10. The cv. Luch 25, after fallow, showed statistically the same weight in the first and second sowing dates, as well as the cvs. Bezenchukskaya Zolotistaya.

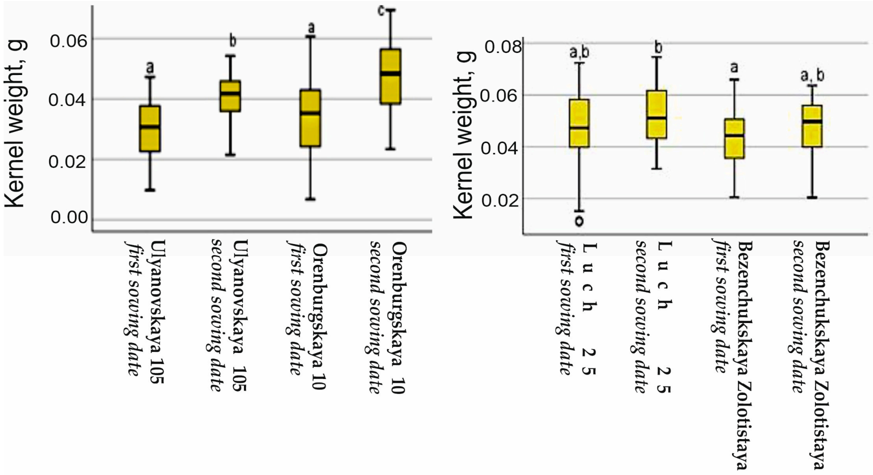


Figure 2. Descriptive statistics of the kernel weight in various wheat cultivars in the first and second sowing dates after plowing (subfigure on the **left**) and fallow (subfigure on the **right**).

3.4. Analysis of the Parameters of Kernels and Their Cross Sections

It was found that the tillage practice had the greatest impact on the kernel parameters of the cv. Orenburgskaya 10 (Table 7). Thus, the kernels had a larger cross-sectional area, perimeter, length, and width when sowing after non-moldboard loosening and after fallow. In cv. Orenburgskaya 30 and Tulaikovskaya Zolotistaya, an increase in the cross-sectional area, length, and width of the kernel was observed when sowing after fallow and non-moldboard loosening.

Table 7. Influence of cultivar, tillage practices, and combination of these factors on kernel parameters.

Cultivar	Tillage Practice	Area of cross Section of Kernel	Perimeter of Kernel	Length of Kernel	Width of Kernel	Length of the Symmetry Axis of the Cut (Thickness of the Kernel)	Length of the Segment from the Bottom of the Cut Hole to the Bottom of the Cut	Total Asymmetry of Kernels
Uchitel	Plowing	a	b	a,b	a	a,b	a	a
	Non-moldboard loosening	a	b	a	a	b	a	a
	Fallow	a	a	b	a	a	a	a
Ulyanovskaya 105	Plowing	a	a	a	a	a	a	b
	Non-moldboard loosening	a	a	a	a	b	a,b	a
	Fallow	b	a	a	b	b	b	a,b
Orenburgskaya 10	Plowing	a	a	a	a	b	a	a
	Non-moldboard loosening	b	b	b	b	b	b	a
	Fallow	b	b	b	b	a	b	a
Bezenchukskaya 210	Plowing	a	a	a	a	a	a	b
	Non-moldboard loosening	a	a	a	a	a	a	a
	Fallow	a	a	a	a	a	b	a
Orenburgskaya 30	Plowing	a	a	a	a	a	a	a
	Non-moldboard loosening	b	a	b	c	b	b	a
	Fallow	b	a	a,b	b	a,b	b	a
Tulaikovskaya Zolotistaya	Plowing	a	a	a	a	a	a	a
	Non-moldboard loosening	a,b	a	a,b	a,b	a,b	a	a
	Fallow	b	a	b	b	b	a	a

The letters indicate significant differences between the indicators, established with Shidak post hoc test for multiple comparisons ($p = 0.05$). Letters a, b, and c are given in increasing order. Orange color indicates the maximum average value of the feature.

A stable increase in the lengths of the perpendiculars from 1a to 5b was observed on the kernel sections of the cv. Uchitel when sowing after plowing and non-moldboard loosening compared to fallow (Table 8). The cv. Ulyanovskaya 105 did not respond to the tillage practices. An increase in the perpendiculars 3a, 3b, 4a, 4b, and 5a occurred on the kernel cuts of the cv. Bezenchukskaya 210 when sowing after fallow and non-moldboard loosening. In addition, a one-sided increase in segments 2b, 3b, and 4b on the kernel sections occurred in cv. Tulaikovskaya Zolotistaya when sowing after similar tillage practices.

Table 8. The influence of cultivar characteristics, soil cultivation methods, and combinations of these factors on the uniformity of kernel filling.

Cultivar	Tillage Practice	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
Uchitel	Plowing	a,b	b	b	b	b	a,b	b	a,b	b	b
	Non-moldboard loosening	b	b	b	b	b	b	b	b	a	b
	Fallow	a	a	a	a	a	a	a	a	a	a
Ulyanovskaya 105	Plowing	a	a	a	a	a	a	a	a	a	a
	Non-moldboard loosening	a	a	a	a	b	a	a	a	a	a
	Fallow	a	a	a	a	a,b	a	a	a	a	a
Orenburgskaya 10	Plowing	b	a,b	a	a	a	a,b	a	a	a	a
	Non-moldboard loosening	b	b	a	b	a,b	a	a	a	b	b
	Fallow	a	a	a	b	b	b	a	a	b	b
Bezenchukskaya 210	Plowing	a	a	a	a	a	a	a	a	a	a
	Non-moldboard loosening	a	a	a	a	a,b	a,b	a,b	a,b	a,b	a
	Fallow	a	a	a	a	b	b	b	b	b	a
Orenburgskaya 30	Plowing	b	b	a	a	a	a	a	a	a	a
	Non-moldboard loosening	a	a	a	a	a	a	a	a	a,b	a
	Fallow	a,b	a	a	b	a	a	a	a	b	a
Tulaikovskaya Zolotistaya	Plowing	a	a	a	a	a	a	a	a	a	a
	Non-moldboard loosening	a	a	a	a,b	a	a,b	a	a,b	a	a
	Fallow	a	a	a	b	a	b	a	b	a	a

Perpendiculars 1a–5b are arranged according to the method of Aniskina et al. (2023) [22]. The letters indicate significant differences between the lengths of perpendiculars on kernel cuts within one cultivar, established with Shidak post hoc test for multiple comparisons ($p = 0.05$). Letters a and b are given in increasing order. Orange color indicates the maximum average value of the feature.

An assessment of the symmetry and uniformity in kernel filling of the left, middle, and right kernels in a simple ear showed that, in the cv. Uchitel, when plowed, there were no differences between the index 1a and 1b; 2a and 2b; 3a and 3b; 4a and 4b; and 5a and 5b, as in the left kernel, both in the middle, and in the right one (Figure 3). If the middle and right kernels were evenly filled when sowing both after non-moldboard loosening and after fallow, then the left kernels looked more asymmetrical when sowing after fallow. Symmetry in the kernel cuts in the cv. Ulyanovskaya was noted only in the middle kernels when sowing after non-moldboard loosening and fallow, and zones 5a and 5b were the most unevenly filled. The fallow conditions were favorable for the cv. Orenburgskaya 10; moreover, non-moldboard loosening showed asymmetry in zones 5a and 5b of the kernel cut. Filling uniformity was noted in the middle kernels of the cv. Bezenchukskaya 210. Sowing after non-moldboard loosening resulted in uneven filling on the kernel cuts in the cv. Orenburgskaya 30 and Tulaikovskaya Zolotistaya.

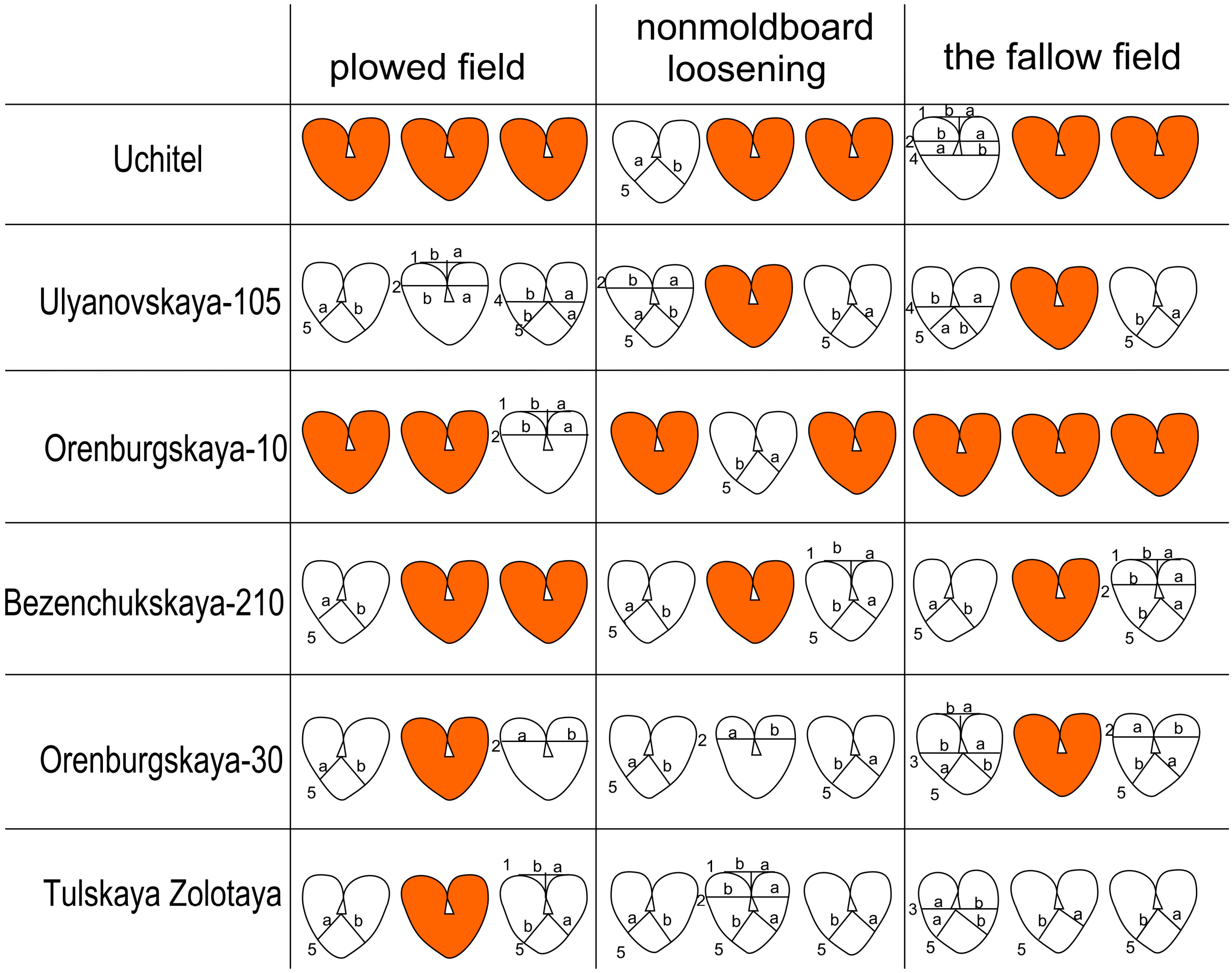


Figure 3. Assessment of asymmetry and uniformity in kernel filling of the left, middle, and right kernels depending on the influence of cultivar characteristics and tillage practices. Perpendiculars 1a–5b are arranged according to the method of Aniskina et al. (2023) [22]. The letters indicate significant differences between the indicators, established by the t-test method (p = 0.05). Letters a and b are given in order of increasing trait value. The cells indicate left, middle, and right kernels. Orange color means close to ideal (symmetrical) shapes of the kernels in the spikelet, depending on the position.

The cv. Ulyanovskaya 105, both on the first and second sowing dates, showed asymmetrically filled right and left kernels in the 5a–5b region, as well as asymmetry in the area of endosperm closure in the middle kernels (Figure 4). However, when sowing on the second date, the middle kernels had time to fill up. The cv. Orenburgskaya 10 showed left and middle kernels more aligned in terms of uniformity on cuts when sowing on the first date. The middle kernels obtained at the second sowing date had a right-sided shift.

In the cv. Bezenchukskaya Zolotistaya, when sown after fallow, only the middle kernels were symmetrical. In the cv. Luch 25, when sown on both dates, the left kernels had asymmetry in the 5a–5b region with a right-sided shift, and the right kernels also had a right-sided shift in the lengths of the perpendiculars in the 2a–2b region.

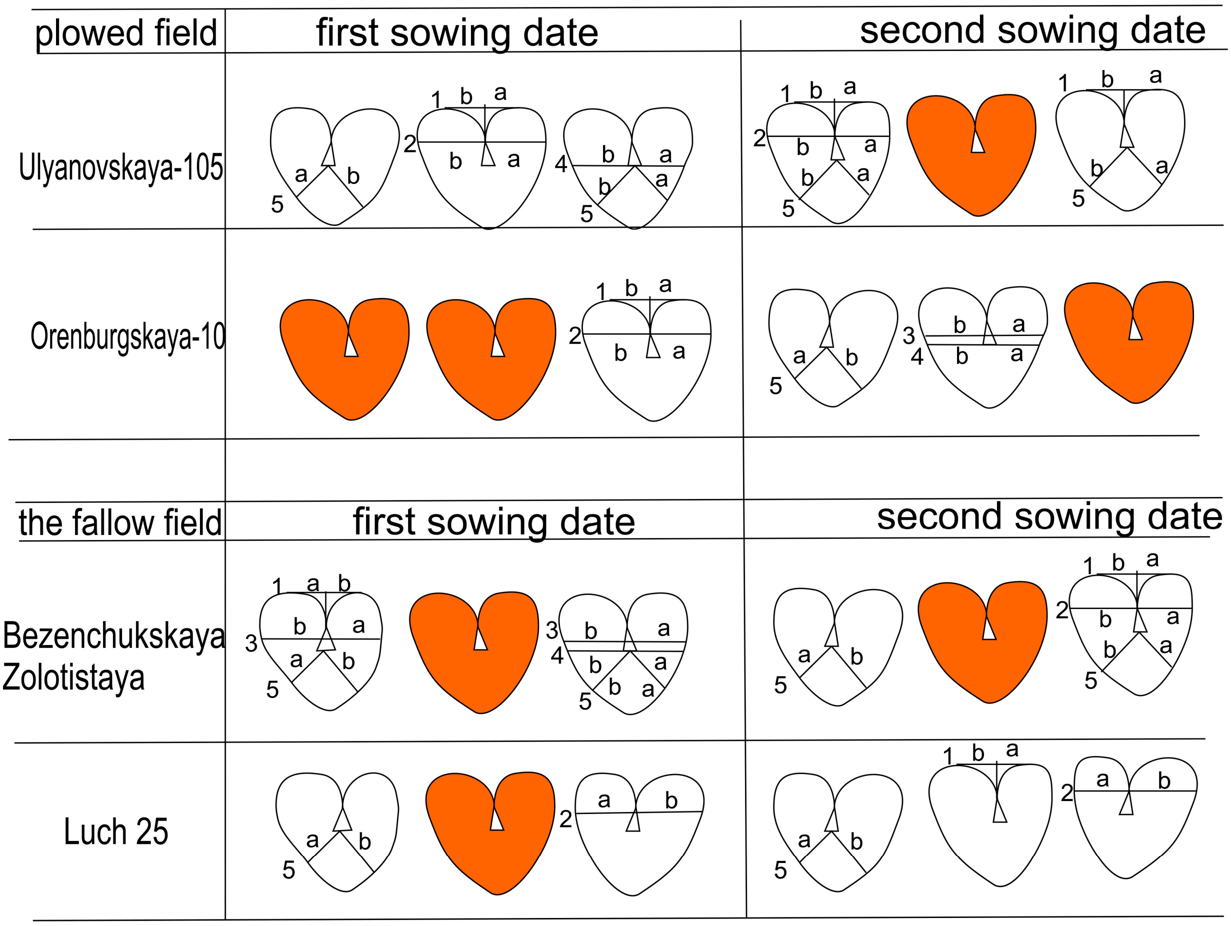


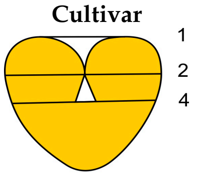
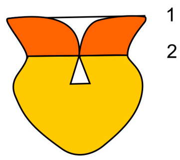
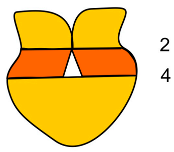
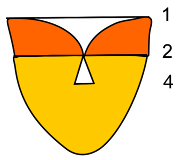
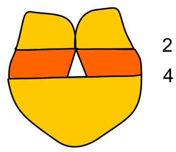
Figure 4. Assessment of asymmetry and uniformity of kernel filling of the left, middle, and right kernels depending on the sowing date. Perpendiculars 1a–5b are arranged according to the method of Aniskina et al. (2023) [22]. The letters indicate significant differences between the indicators, established by the t-test method ($p = 0.05$). Letters a and b are given in order of increasing trait value. The cells indicate left, middle, and right kernels. Orange color means close to ideal (symmetrical) shapes of the kernels in the spikelet, depending on the position.

3.5. Kernel Fullness Analysis

The cvs. Ulyanovskaya 105, Bezenchukskaya Zolotistaya, and Tulaikovskaya Zolotistaya demonstrated stable kernel filling during sowing after all tillage practices (Table 9). For the cv. Uchitel, plowing turned out to be more favorable, since, when sowing after non-moldboard loosening, 3% of the kernels had a filling index below 0.75, and, after fallow, 16% of the kernels showed such an index. The kernels of the cv. Orenburgskaya 10 had a crease in the area of line 2 (the widest part of the standard kernel), so much so that 28% of the kernels obtained in the wheat plants grown after plowing and 21% of the kernels obtained after fallow looked rather puny (the 2/4 ratio is less than 0.75).

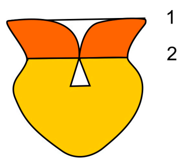
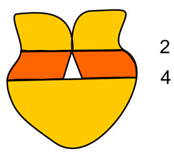
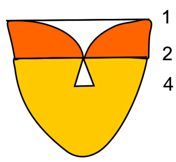
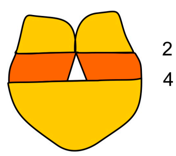
When considering the influence of sowing dates, it can be noted that, in the cv. Ulyanovskaya 105, the kernels were equally well-filled (Table 10). While in the cv. Orenburgskaya 10, the kernels were obtained only from plants grown after sowing on the second date, after sowing on the first date, up to 28% of the kernels had a 2/4 ratio index below 0.75 and more than half (53%) had an index below 0.9.

Table 9. Percentage of kernels deviating from the reference.

Cultivar  standard ratios of kernel section line lengths 2/1 > 1 2/4 > 1	**Tillage Practice**	 2/1 < 0.75	 2/4 < 0.75	 2/1 < 0.9	 2/4 < 0.9
Uchitel	Plowing Non-moldboard loosening Fallow	0-0-0 0-0-3% 0-0-0	0-0-0 0-0-3% 9%-0-7%	0-0-0 0-0-3% 0-0-2%	5%-10%-7% 3%-3%-12% 23%-0-20%
Ulyanovskaya 105	Plowing Non-moldboard loosening Fallow	0-0-0 0-0-0 0-0-0	0-0-0 0-0-0 0-0-0	0-0-0 0-0-0 0-0-0	0-2%-0 0-0-0 2%-0-0
Orenburgskaya 10	Plowing Non-moldboard loosening Fallow	0-0-2% 0-0-0 0-0-0	3%-14%-11% 0-3%-0 14%-5%-2%	0-0-2% 0-0-0 0-0-0	11%-17%-25% 3%-5%-24% 24%-12%-21%
Bezenchukskaya 210	Plowing Non-moldboard loosening Fallow	0-0-0 0-0-0 0-0-0	0-0-2% 0-0-0 0-0-0	0-0-0 0-0-0 0-0-0	0-3%-3% 0-0-0 0-2%-0
Orenburgskaya 30	Plowing Non-moldboard loosening Fallow	0-0-0 0-0-0 0-0-0	2%-2%-0 0-2%-0 0-0-0	0-0-0 0-0-0 0-0-0	8%-6%-2% 0-2%-0 0-7%-0
Tulaikovskaya Zolotistaya	Plowing Non-moldboard loosening Fallow	0-0-0 0-0-0 0-0-0	0-0-0 0-2%-0 0-0-0	0-0-0 0-0-0 0-0-0	0-5%-0 0-2%-0 0-2%-2%

For the reference kernel, the ratio of the line of the middle part (line 2) to the line of the upper part of the cut (line 1) and to the line under the crease cavity (line 4) is greater than one. The percentages of deviations for left-middle-right kernels in a simple ear are indicated through a hyphen.

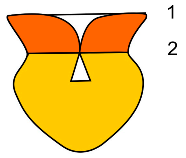
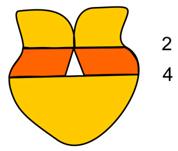
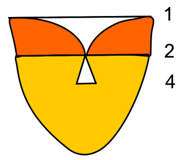
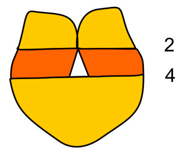
Table 10. Percentage of kernels deviating from the reference at two sowing dates after plowing.

Cultivar	**Tillage Time**	 2/1 < 0.75	 2/4 < 0.75	 2/1 < 0.9	 2/4 < 0.9
Ulyanovskaya 105	First Second	0-0-0 0-0-0	0-0-0 0-0-0	0-0-0 0-0-0	0-6%-0 0-0-0
Orenburgskaya 10	First Second	0-0-2% 0-0-0	3%-14%-11% 0-0-0	0-0-2% 0-0-0	11%-17%-25% 0-0-0

The kernels have been considered in which the ratio of the line of the middle part (line 2) to the line of the upper part of the cut (line 1) and to the line under the crease cavity (line 4) is greater than one. The percentages of deviations for left-middle-right kernels in a simple ear are indicated through a hyphen.

The cvs. Bezenchukskaya Zolotistaya and Luch 25 demonstrated a high degree of kernel filling (plumpness) (Table 11).

Table 11. Percentage of kernels deviating from the reference at two sowing dates after fallow.

Cultivar	Sowing Date	 2/1 < 0.75	 2/4 < 0.75	 2/1 < 0.9	 2/4 < 0.9
Bezenchukskaya Zolotistaya	First Second	0-0-0 0-0-0	0-0-0 0-0-0	0-0-0 0-0-0	0-0-0 0-2%-2%
Luch 25	First Second	0-0-0 0-0-0	0-0-0 0-0-0	0-0-0 0-0-0	0-2%-0 2%-2%-0

The kernels have been considered in which the ratio of the line of the middle part (line 2) to the line of the upper part of the cut (line 1) and to the line under the crease cavity (line 4) is greater than one. The percentages of deviations for left-middle-right kernels in a simple ear are indicated through a hyphen.

3.6. Comparative Hyperspectral Analysis of Kernels

An evaluation of the relationship between the brightness of each layer of the hyperspectral date (layers 392 to 1032, with a step of 2 layers) and the area, perimeter, length, width, and thickness of the kernel (axis of symmetry), as well as an evaluation of the kernel cut parameters (perpendiculars 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, and 5b; the depth of the funnel and the distance from the kernel cavity to the bottom of the kernel) showed the presence of significant correlations, but they did not exceed r = 0.4. Thus, significant feedback between the kernel width with layers 444 to 532 (blue-green spectrum) with r = 0.4 and with layers 534 to 682 (yellow-red spectrum) with r = 0.3 was noted. Perpendicular 1a had an r of up to −0.3 with layers 516–600 (green-yellow spectrum) and up to −0.2 with layers 602–610 (orange spectrum). Perpendicular 4a had an r of up to −0.2 in the yellow-orange spectrum (layers 556–600). A direct significant relationship at the level of r = 0.3 was noted in perpendiculars 2a and 2b with the layers of the near-infrared spectrum (layers 850–1032).

Color maps are a powerful data visualization tool that allows us to convert data values into colors. They are divided into several classes, including sequential, divergent, and cyclic, each of which serve a different purpose for visually representing data. With color maps, we can classify and separate data in a much more efficient way.

It was found that the kernels of the cv. Orenburgskaya 10 obtained from the plants grown during the second sowing date were significantly different from the kernels of the first sowing date and exceeded them by two brightness units in the green, yellow, and orange layers of the spectrum. This was also observed in the anthocyanin index (Figure 5).

The kernels obtained from the wheat plants grown after two sowing dates within the cvs. Luch 25, Bezenchukskaya Zolotistaya, and Ulyanovskaya 105 did not differ significantly. When comparing the spectra of the hard wheat cultivars, it turned out that the cv. Orenburgskaya 10 had the highest brightness in layers 430–540. The cv. Bezenchukskaya Zolotistaya did not differ from it or from the cv. Luch 25 in the spectrum layers 430–470 and 500–540, respectivley. According to the yellow spectrum, all three cultivars of the hard wheat were the same. The cultivar with the highest brightness in the orange, red, and near-infrared spectrum was the cv. Bezenchukskaya Zolotistaya, and the cv. Orenburgskaya 10 did not differ from it or from the cv. Luch 25 in the orange and red spectrum.

The hyperspectral data were processed and analyzed using various algorithms. In this case, Figure 5a,b shows data on the anthocyanin index Anc. We consider it necessary to indicate that not only the method of obtaining and processing data is important, but also their final visualization. Figure 5a used the common color map "Red & Green", while Figure 5b used a color map called "Jet". Both cases present visualizations of the same data sets; however, using color maps with more colors may be more visual in some cases. By comparing enlarged images of a symmetrical, well-filled mature kernel and a wrinkled, puny kernel using an "ideal model", it is possible to determine the differences in the symmetry of the right and left parts of the kernel. However, this requires the development of automatic visualization technologies, possibly using specially created neural networks.

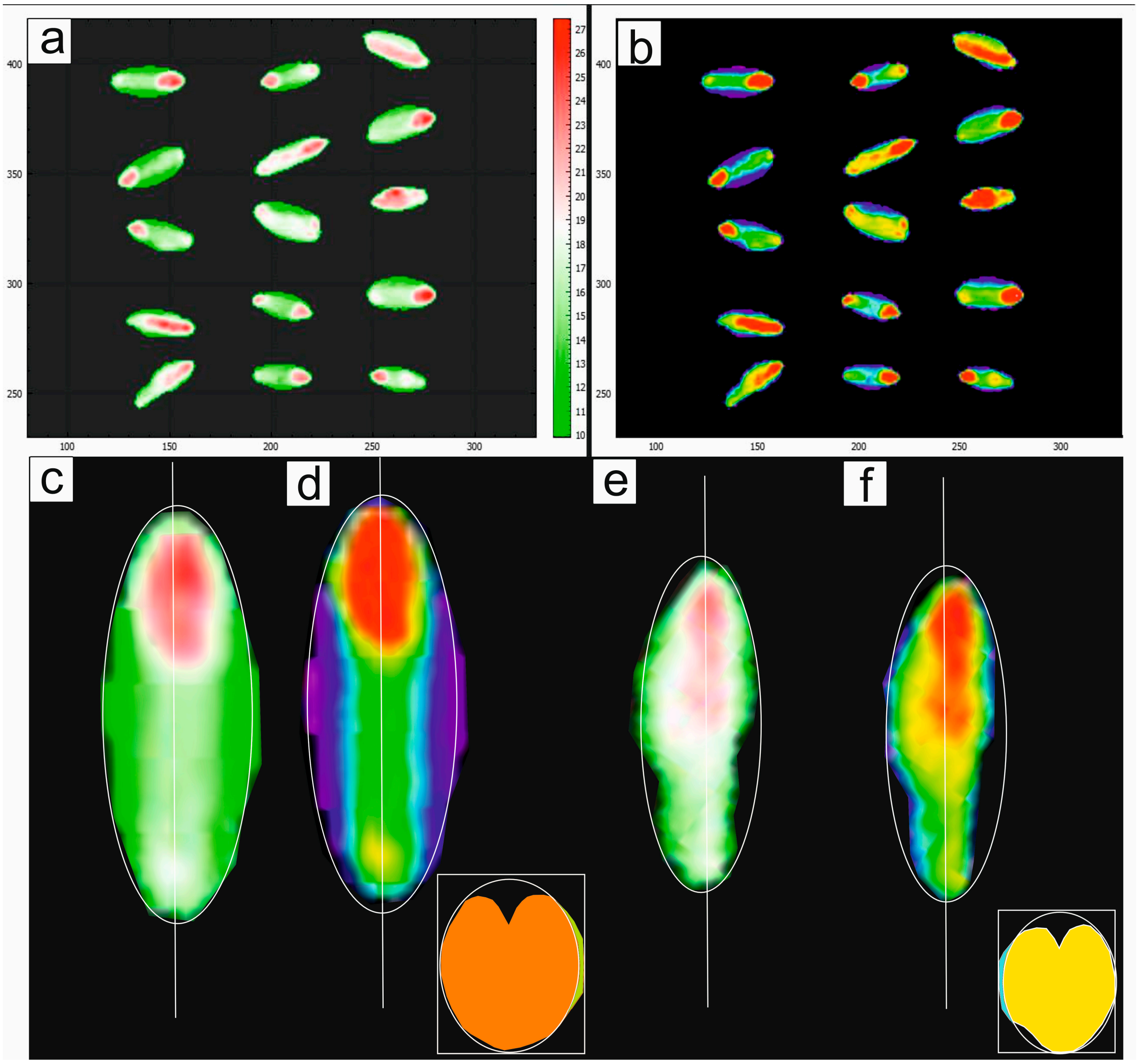


Figure 5. Hyperspectral images of kernels of the cv. Orenburgskaya 10 obtained from plants grown at the first sowing date with color map "Red & Green" (**a**), and the second sowing date used a color map "Jet" (**b**) with an overlay of the anthocyanin content index. Designations: hyperspectral images with an overlay of the ellipse of the "ideal kernel shape" and the axis of symmetry (**c**–**f**); images with color map "Red & Green" (**c**,**e**); images with color map "Jet" (**d**,**f**); orange image: a section of the wide part of the "symmetrical kernel;" yellow: a section of the wide part of the "puny asymmetrical kernel".

4. Discussion

The formation of the ear and spikelets in wheat occurs inside the most protected part of the plant, surrounded by leaves, and is associated with the transformation of the meristem into a rudimentary ear during the stem elongation (or booting) stage, that is, as it grows and transitions from the tillering stage (when the meristem is still only tubercle) to the heading stage (when the ear and its individual structural elements are fully formed) [31]. Thus, damage to development at the booting stage can lead to a decrease in initiation and a stopping of the formation of developed spikelets and flowers. When plants are damaged at a later stage of heading and flowering, adverse effects cause a disruption of the development of the flower organs, including both maternal parts and the tissues of the new sporophyte. This is due to the premature death of the integument and its deformation. There is also a disruption of the formation of the aleurone layer and endosperm. At the same time, the tissues of the new sporophyte, the triploid endosperm, and the internal tissues of the maternal integument of the complex wheat fruit (caryopsis or kernel) remain alive [21,32]. The tissues of the new sporophyte, the embryo, remain the most protected, which makes it possible to effectively use (if necessary, to consolidate a valuable trait) the immature seeds for accelerated selection [33].

In this study, it was found that plowing contributes to a significant elongation of the ear in the soft wheat cvs. Uchitel, Ulyanovskaya 105, and Orenburgskaya 30. Sowing hard wheat cvs. Orenburgskaya 10 and Bezenchukskaya 210 after non-moldboard loosening resulted in a shorter ear length. The cv. Uchitel, when sown after fallow, had significantly fewer fertile spikelets than that sown after plowing and non-moldboard loosening. Here, the maximum fertility results took place throughout this study, since the cv. Uchitel generally responds poorly to improved agricultural conditions, and the conditions of the 2022 growth season were not the most favorable for it [34]. The cv. Ulyanovskaya 105 had the maximum number of fertile spikelets for all of the tillage practices. This was due to the genotypic characteristics of the cultivar, such as the following: it has a longer growing season compared with the other studied cultivars; the interphase period from tillering to booting is 2 days longer; and the period from booting to heading is 2–3 days longer, which allows this cultivar to increase the number of fertile spikelets. The conditions for non-moldboard loosening of plowed land and fallow turned out to be the most favorable in the year of research in terms of productive moisture content. The difference in favor of these tillage practices compared to plowing was 28–33 mm during the sowing period and 23–27 mm at the heading stage of the wheat, which contributed to the creation of favorable conditions for ear formation. It should be taken into account that, in the study area, the main factor limiting the productivity of the cultivated crops was the amount of productive moisture in the soil. Thus, in the cvs. Ulyanovskaya 105, Bezenchukskaya 210, and Tulaikovskaya Zolotistaya, the maximum number of kernels was observed precisely after the non-moldboard loosening and fallow. The cvs. Orenburgskaya 30 and Tulaikovskaya Zolotistaya showed a large number of immature kernels. These results are consistent with Woźniak's research with plowing [35,36]. Cultivar variability can be considered the result of a long-term, unwitting experiment in which environmental conditions, soil cultivation traditions, and human factors impact its microevolution, with the development of variants for both adaptive and qualitative characteristics [37].

The growing conditions for the wheat after sowing on the second date were more favorable in comparison with the conditions after the first date in terms of productive moisture content. When sowing on the first date, its amount in the 1-m soil layer was 105 mm, and, when sowing on the second date, it was 148 mm. During the wheat heading period, the amount of available moisture when sowing on the first date was 46 mm, and, when sowing on the second date, it was 75 mm. By the end of the growing season, in areas where the wheat was sown on the first date, 5 mm of productive moisture remained, and, in areas sown on the second date, 12 mm remained. This moisture content determined the response of the plants to an increase in the amount of moisture by increasing the total number of kernels in the ear, reducing the number of immature kernels, and increasing the mass of the individual kernels. Accordingly, when sown on the second date, after plowing, the total number of kernels and the number of middle kernels increased, while the number of immature kernels decreased in the cvs. Ulyanovskaya 105 and Orenburgskaya 10. In the cvs. Bezenchukskaya Zolotistaya and Luch 25, the number of immature kernels was reduced during growth after the second sowing date. Also, in the cvs. Ulyanovskaya 105 and Orenburgskaya 10, the weight of the kernel increased at the second sowing date. In addition, in the cvs. Luch 25 and Bezenchukskaya Zolotistaya, at the second sowing date after fallow, the weight of the kernel did not increase, which was due to the peculiarities of the genotype response. It follows that our results are consistent with the studies suggesting that the parameters of the kernel are critically affected by the availability of water, reducing the efficiency of the transport of metabolites from the flag leaf [38–41].

The environmental conditions can lead to a decrease in the filling of the cells in the triploid endosperm tissues of the wheat kernel, especially important environmental factors such as temperature and access to water [24,39,41]. The daughter tissues of wheat kernels (embryo and endosperm) formed after fertilization begin to actively divide and grow (forming a new gametophyte), and the nucellus tissue cells die by programmed cell death [42]. The period of kernel filling is maximally extended at a temperature of 15–20 °C, after which the greatest degree of starch accumulation in the seeds is achieved, which leads to the production of good-quality wheat. Furthermore, any deviation from the optimal temperature leads to a change in the physiology of the wheat. For example, an increased temperature shortens the kernel development period, promotes the accumulation of dry matter, and leads to dehydration [43], whereas a decrease in temperature leads to an extension of this period.

During the filling stage of the wheat kernel, the three successive periods can be distinguished. During the first period (the first 1–2 weeks), the rate of dry matter accumulation in the kernel is not high, but the ability of the kernel to accumulate starch is established [14,23]. The second period, the "linear phase", lasts for most of the period of kernel filling, during which the rate of kernel growth is almost constant, which corresponds to the stage of the maximum rate of accumulation of kernel biomass if there is an excess of assimilates for the kernel needs [44–46]. During the third period, the "maturity phase", the rate of starch deposition decreases rapidly [47]. Our study showed that the degree of kernel filling is more likely to be related to the varietal characteristics of wheat than to the timing of tillage.

The kernel filling process is carried out as follows: the precursors of storage compounds are transported from the mother plant through the vascular tissues of the pericarp in the ventral region of the kernel [23,24,48]; the ventral region forms a fold (crease), which, in wheat and barley, is quite deep compared to that of other cereals [49]; the storage compounds move through the chalaza and the nucellar projection cells move to aleurone transport cells and endosperm starch cells [50]; and the cavity separating the endosperm of the wheat ends with a group of transfer cells, some of which belong to the nucellus tissue. These cells transport nutrients, which then form starch reserves in the endosperm cells and protein bodies in the aleurone layer [50–53]. In our work, it was found that, in the kernels of the cv. Uchitel plants, which were sown after plowing and non-moldboard loosening, there was a uniform distribution of storage substances filling the endosperm. According to the analyzed parameters of the ears and kernels, the cv. Ulyanovskaya 105 did not significantly respond to the tillage practices. In the soft wheat cvs. Orenburgskaya 30 and Tulaikovskaya Zolotistaya, which were sown after plowing, the presence of predominantly symmetrical middle kernels was noted. Therefore, the uniformity in the process of deposition of reserve substances is presumably related to the varietal characteristics of wheat plants.

The starch accumulation in the kernels is directly related to the yield and quality of the wheat [54]. Its content in the cell reaches up to 70% [3], therefore, the filling of the endosperm cells with starch can affect the shape of the kernel. At the initial stage of wheat kernel development, approximately 4–5 days after flowering, A-type starch with large granules with a diameter of 20–50 μm is formed in the amyloplasts. B-type granules appear 10–12 days after flowering, and their size continues to increase to 9 μm until 21 days after flowering [55,56]. Sometimes, a third type of granule is identified—C-type; moreover, such granules are formed at the final stage of kernel development and are very small [56]. The size ratio of the granular structure affects the overall quality of the starch. The temperature levels and the availability of water and nitrogen sources are the main environmental factors influencing the rate of starch accumulation in wheat [38,40,54]. As reported by Dai et al. (2008), growing wheat without irrigation increases the volume and percentage of the surface area of B-type starch granules and decreases the percentage of A-type starch granules in wheat grain, in contrast to the irrigated variant [57].

The hyperspectral image analysis of cvs. Luch-25, Bezenchukskaya Zolotistaya, and Ulyanovskaya 105 wheat kernels did not reveal any significant differences between the accessions from the plants sown on different dates. However, the kernel analysis of the three hard wheat cultivars revealed significant differences in the spectra between the cultivars for this indicator. This may be explained by the fact that the color of the seed coat depends on the production of pigments in the seed coat cells, which are influenced by both genetic factors and the environmental conditions [58–61]. When testing the difference between the kernels that differed in that they were obtained from wheat plants sown on different dates, it was found that, within a cultivar, the genetic diversity did not have as significant of an effect as when comparing the average brightness values of the spectra of the different cultivars [62].

Various researchers involved in improving wheat yield have focused on yield components such as kernel weight and length, ear length, number of kernels per ear, number of spikelets, and their correlation with plant height, chlorophyll content, and so on [63,64]. In addition, sufficient attention is paid to issues of resistance to biotic and abiotic factors [65]. We also see prospects for further research that include parameters of kernel plumpness (fullness). In particular, this can help us to improve the efficiency and predictability of these studies, which remain routine and labor-intensive.

This article examines in detail how the symmetry of the cross-sectional shape of all types of grains in an ear changes under the influence of the soil cultivation methods (Table 8). We have examined these same factors more carefully on the outer grains (left and right grains in a simple ear) and on the middle grains. It turned out that, in the cv. Uchitel variety, all types of grains have a symmetrical shape in the plowed field and in Orenburgskaya-10 in the fallow field. Also, the chance of selecting a model kernel with an ideal symmetrical shape was significantly higher in the cvs. Uchitel and Orenburgskaya-10 with different soil tillage methods (Figure 3). A high level of asymmetry in 10 parameters (cut lengths of the kernels from 1a to 5b) was found in the cvs. Ulyanovskaya-105, Orenburgskaya-30, and Tulaikovskaya Zolotaya; therefore, in further breeding work, attention should be paid to improving the shape of these varieties, since the asymmetric filling of the grains leads to a decrease in the yield quality of products in terms of the protein and starch content of A-type starch granules.

5. Conclusions

This study is the first to examine in detail the dependence of kernel filling capacity on cultivar characteristics, soil tillage regime, and sowing time. The methodological approaches, indicators, and parameters for an analysis characterizing the unstable development and asymmetric deposition of storage reserves in tissues during the formation of the most valuable part of the wheat fruit (kernel), consisting of the aleurone layer and the triploid endosperm, are proposed. These methods may be useful for future targeted studies applying phenotypic analysis and the further development of digital methods. In the future, the identified differences can produce good results in the development of high-throughput analysis methods (automated phenotyping and phenomics) and will help us to significantly reduce the time of accession analysis, taking into account the fluctuating asymmetries that are currently not considered. This method may also be promising for predicting the deposition of starch, protein, and secondary metabolites under various growing conditions.

The size and shape affect the quantitative and qualitative characteristics of the endosperm of wheat kernels and, consequently, the quality of flour. Breeding methods and agronomic practices can improve the kernel size indicators, but the issue of shape symmetry remains open. Our study showed that the soil tillage methods influenced the uniformity of cell filling in different zones of the endosperm. The symmetry of the grain is more likely to be related to the varietal characteristics of wheat than to the timing of sowing (i.e., this hypothesis has not been confirmed, at least for the varieties studied), although this factor affects such indicators as changes in the total number of kernels in an ear, the weight, and the number of unripe kernels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/sym16050548/s1, Table S1: Weather conditions of the growing season of 2022 in comparison with long-term observations; Table S2: Duration of interstage periods in the growing season of spring wheat cultivars in 2022; Figure S1: Evaluation schema of the kernel indicators depending on the cultivar and tillage practice; Figure S2: Evaluation schema of the kernel indicators depending on the sowing date, cultivar, and tillage practice.

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